

treating said DNA to form single-stranded complementary strands;  
adding at least three [paired] pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand and all primers having similar melt temperature characteristics;

annealing the at least three pairs of primers to their complementary sequences all primers being subjected to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair[, wherein the extending step includes the condition selected from the group consisting of increased enzyme, increased extension times and combination thereof, and wherein said increase is effective to simultaneously extend all the primers];

separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded molecules by repeating, at least once, said annealing, extending and separating steps; and

identifying said amplified extension products from each different sequence.

Add New Claims:

18. The method of claim 1, wherein said at least three pairs of primers have Tms such that the lowest Tm and the highest Tm vary by no more than <sup>3.0</sup><sub>8.5</sub>° C.

19. The method of claim 1, wherein said at least three pairs of primers have Tms such that the lower Tm of each pair varies from the lower Tm of each other pair by no more than <sup>4.0</sup><sub>4.5</sub>° C.

20. (Amended) A method for simultaneously detecting at least three DNA sequences, comprising the steps of:

adding to a common reaction vessel containing a sample mixture of at least three distinct, target sequences in single-stranded form, at least three pairs of oligonucleotide primers, each pair specific for a different sequence, one primer of each pair substantially complementary to a part of the sequence in the sense-strand and the other primer of each pair substantially complementary to a different part of the same sequence in the complementary anti-sense strand; wherein the primers all have similar melt temperature characteristics;

annealing the at least three pairs of primers to their complementary sequences, all primers being subject to the same reaction conditions;

simultaneously extending said at least three pairs of annealed primers from each primer's 3' terminus to synthesize an extension product complementary to the strands annealed to each primer, said extension products, after separation from their complement, being capable of serving as templates for the synthesis of an extension product from the other primer of each pair;

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separating said extension products from said templates to produce single-stranded molecules;

amplifying said single stranded target sequences by repeating, at least once, said annealing, extending and separating steps; and

identifying whether amplified extension products have been synthesized from each different sequence, as a <sup>result</sup> ~~measure~~ of the presence or <sup>absence</sup> ~~amount~~ of each target sequence.

### REMARKS

Claims 1-8 and 18-20 are pending in the application. Claim 1 has been amended. Claims 18-20 have been added. No new matter has been introduced. Support for the amendments to claim 1 is found on page 16 in Example 1 of the Specification and in new Table 1 submitted with this response. The melt characteristics are inherent to the oligonucleotides.

### Section 112 New Matter

The examiner contends that the addition to claim 1 of the limitation "all primers having similar melt characteristics" constitutes new matter. Applicants disagree. The examiner does not dispute that Table 1 (p. 20) discloses the sequences of seven distinct primer pairs. Each primer possesses an inherent property known as its melt temperature or T<sub>m</sub>. These are given in the amended Table 1.<sup>1</sup> The physical addition to an application of a feature that is inherently

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<sup>1</sup>The values given in the far right columns of amended Table 1 represent the predicted or theoretical T<sub>m</sub>s as calculated without undue experimentation by the algorithm of the most commonly used method. K. J. Breslauer, et al. *Predicting DNA duplex stability from the base sequence*, Proc. Nat. Acad. Sci. USA 83:3746-